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REVERSIBLE ARREST OF UPTAKE OF WATER FROM SUBSATURATED ATMOSPHERES BY THE FIREBRAT, *THERMOBIA DOMESTICA* (PACKARD)

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SUMMARY

1. A novel experimental technique incorporating a double-chambered array, which allows the independent and reversible exposure of head and tail ends of the insect to the surrounding atmosphere, without otherwise altering the constraints on the insect, is described.

2. Using this technique, it has been possible to demonstrate that rapid uptake of water from subsaturated atmospheres occurs in *Thermobia* only when the tail-end is exposed.

3. This effect is immediately and completely reversed when the tail-end is shielded from the atmosphere, and can be restored again on re-exposure of the tail-end, as shown repeatedly during electrobalance recordings.

4. The relative humidity within the closed head-end chamber slowly rises, but within the closed tail-end chamber it falls, to approximately 50% (the critical equilibrium humidity) with the desiccated insects and to 60–65% with hydrated insects, whilst the anal valves show movements indicating the operation of the rectal uptake mechanism.

5. These findings are compatible with the anus being the avenue for the passage of water into the insect from the atmosphere.

INTRODUCTION

The involvement of the rectum in the uptake of water from subsaturated atmospheres in insects has been inferred from experiments in which blockage of the anus prevented uptake in *Thermobia* (Noble-Nesbitt, 1970*a*, *b*). Further confirmation came from preliminary studies on *Tenebrio*, in which uptake was also arrested by occlusion of the anus (Noble-Nesbitt, 1970*a*, *b*). In this insect, uptake is possible once more after removal of the blockage (Noble-Nesbitt, 1973), an operation which could not be carried out on the more fragile *Thermobia* without causing damage to the insect. These results obtained with *Tenebrio* have recently been supported by the ligature experiments of Wicken & Winston (1975) on this insect. The complex fine-structure of the terminal region of the rectum in *Thermobia* was cited as further evidence supporting the view that the rectum is the site of uptake (Noble-Nesbitt, 1970*a*, *b*). These preliminary observations had shown that there was a close juxtaposition of mitochondria and the apical plasma membranes of the epithelial cells in a very dense and regular

array. More recently, Noirot & Noirot-Timothée (1971) have described in detail the structure of this region of the rectum in *Thermobia*. Further work in progress has shown that changes occur in this fine structure under varying environmental conditions involving desiccation and rehydration, and this will be published in full elsewhere (see Noble-Nesbitt, 1973, for a preliminary report). These results further substantiate the direct involvement of the posterior rectal region in the uptake of water from the atmosphere.

It has been suggested that an alternative explanation may account for this apparent involvement of the rectum in uptake (Okasha, 1971). This alternative explanation is based on the possible interruption of proprioceptive, or other, feedback controlling the volume regulatory system which is known to operate in *Thermobia* (Noble-Nesbitt, 1969; Okasha, 1971, 1972), caused by the experimental application of wax over the anus. No direct experimental results support this suggestion; on the contrary, control experimental procedures included in the original investigation appeared to exclude this possibility, though it may be argued that blockage of the anal aperture *per se* could affect the volume regulatory system. In this case, the control application of wax to other parts of the body would not provide a sufficient control. What is required is some means of alternately exposing the anal aperture to, or shielding it from, an atmosphere of high humidity, without otherwise altering the constraints on the insect.

The situation in ticks is different however. Anal occlusion does not prevent uptake (McEnroe, 1973; Knülle & Devine, 1972). Recently, Rudolph & Knülle (1974) have shown that a specific site, the buccal cavity and associated salivary glands, is implicated in uptake by ixodid ticks. This avenue had been eliminated as a possiblity in the earlier experiments with *Thermobia* in which occlusion of the mouth failed to stop uptake (Noble-Nesbitt, 1970*a*, *b*).

A further series of investigations has thrown more light on these problems. A brief outline of some of the main results of these investigations has been given in a recent review article (Noble-Nesbitt, 1973); together they provide considerable additional evidence that the rectum is the site of uptake in *Thermobia*. This report deals with one of the main lines of investigations; experiments in which it was possible to follow the effects of reversible exposure of the anus to high humidity. Other aspects of the investigations will be the subjects of separate detailed reports.

MATERIALS AND METHODS

Stock cultures of the firebrat, *Thermobia domestica* (Packard) (a junior synonym for *Lepismodes inquilinus* Newman, but retained for convenience in the present context – see Noble-Nesbitt, 1969), were kept at 37 °C and in 83 % relative humidity (R.H.), without access to liquid water. The insects were fed on an equal mixture of protein rich 'Baby Oats' and 'Baby Wheat' ('Twin Pack' Baby Cereal, A. & R. Scott Ltd, Edinburgh) to which a small amount of dried yeast was added.

Some specimens from these stock cultures were kept in individual feeding tubes, a procedure which minimizes abrasion (Beament, Noble-Nesbitt & Watson, 1964) and allows more rapid growth. For use in the experiments, adult insects of suitable size and condition were taken either direct from the stock cultures or from individual feeding tubes.

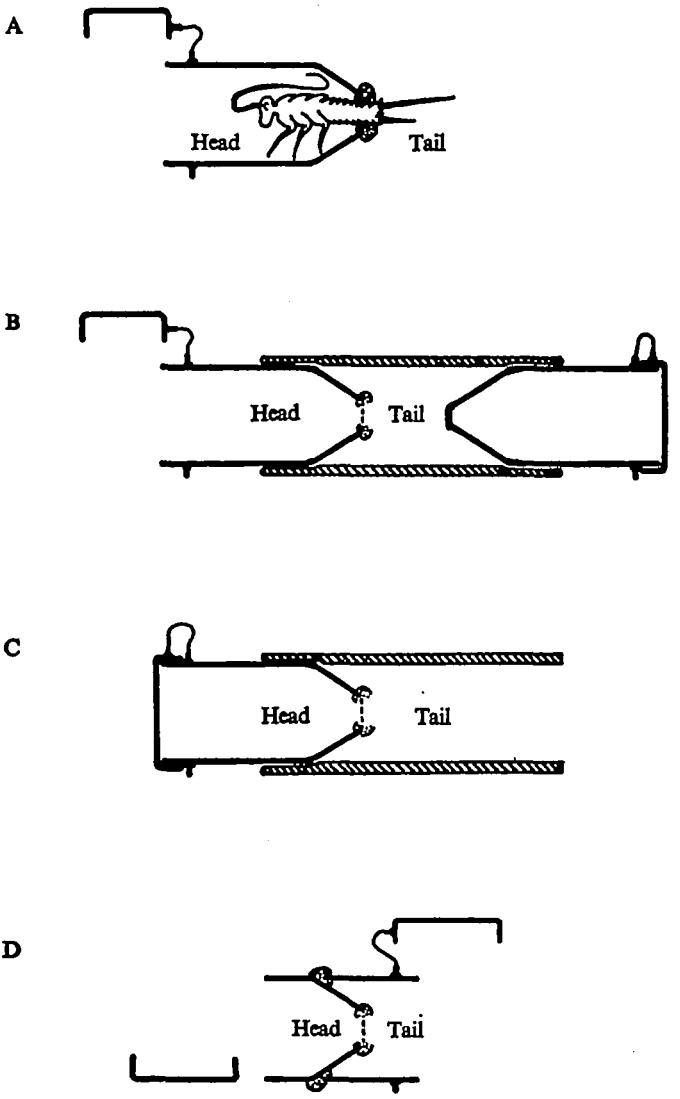

Fig. 1. The double-chambered array. (A) Basic component, formed from a capped polythene capsule. (B) Complete array, showing head-end open. (C) Complete array, showing tail-end open. (D) Modification for use on electrobalance. Cross-hatching indicates polythene or perspex tubing, and stippling indicates wax used to seal the insect in place and to cement the parts of the array together. The position of the insect is indicated in (A).

During the course of the experiments, the insects were confined individually without food in suitably marked, clean glass vials, after being weighed to record their initial weights. Desiccation was carried out over dry granular calcium chloride at 37 °C and rehydration was carried out by exposing the insects to 83 % R.H. (maintained by saturated potassium chloride solution) at 37 °C, unless stated otherwise. The insects were briefly removed from the experimental conditions for weighing, which was carried out on an analytical balance accurate to 0·1 mg.

Experiments involving continuous weight recording were conducted in a special

chamber attached to a Cahn RG Electrobalance connected to a suitable potentiometric recorder as described previously (Noble-Nesbitt, 1969).

In the experiments involving the separation of the atmospheres in contact with the anterior and posterior ends of the insect, a novel technique was employed. A capped polythene capsule as used in the embedding of specimens for electron microscopy, had its tip removed so as to provide an aperture of similar diameter to the girth of the insect. Whilst being held under carbon dioxide anaesthesia for 1–2 min, the insect was waxed into this holder, with its posterior end protruding from the capsule tip. Just-molten paraffin wax with a melting point of 54 °C was used to effect the seal. The arrangement is shown in Fig. 1 A. The amount of the insect left protruding from the capsule could be varied, but for the experiments described here, the seal was made around the posterior part of the abdomen. The cap could be used to seal the chamber at the 'head-end'. The tip of the capsule fitted tightly into polythene or perspex tubing, the other end of which could be stoppered, forming a sealed 'tail-end' chamber. The two separated compartments so formed could be held open, or closed, independently, without affecting the insect in any other way (see Fig. 1 B, C). These compartments were of small volume (approximately 0·5 ml), so the amount of water vapour which could be held in them when closed was negligible (a maximum of approximately 0·02 mg) and could therefore be ignored, either as a drain on the insect's water resources, or as a source of water for it, during the experiment. The insect was weighed just prior to insertion in the holder. Immediately after insertion, the basic component of insect plus capsule plus wax was weighed, giving a measure of the combined weight of the capsule and wax. This basic component was used for subsequent weighings as outlined in the relevant sections below. Insertion into the polythene or perspex tubing forming the 'tail-end chamber' was done as required. The basic component also formed a convenient holder for the insect.

For experiments involving continuous recording of weight on the electrobalance, the array was further modified in order to limit its total weight. The capped end of the capsule was cut off, so shortening the anterior chamber, which could still be closed using a detached cap. The cut-off portion with cap still attached, was used to form the posterior chamber. It was sealed into position with wax after the insect had been sealed into place in the anterior chamber. The arrangement is shown in Fig. 1 D.

To determine the humidity with which the insect came into equilibrium, the method described by Winston & Bates (1960) was used. Individual insects were confined in small sealed tubes, of approximately 1 ml volume. A small crystal of an appropriate salt was also placed in the sealed tube, on a polythene plate out of reach of the insect, and observed microscopically for evidence of gain or loss of water. A range of salts was used to determine the R.H. as accurately as possible. These determinations were carried out in a constant temperature room set at 21 °C, in which the R.H. was about 50%. In some cases, the salt crystal was moistened before insertion, or humid air introduced into the tube, to check whether the humidity within the tube actually could be lowered from a higher value. A similar technique was employed to check the R.H. produced in the sealed head and tail chambers of the double-chambered array. For this purpose, clear perspex tubing was used to form the tail chamber and to extend the head chamber. This allowed the microscopic observation of the salt crystals and also of the insect.

RESULTS

(1) *Preliminary experiments and observations*

(*a*) *Effects on the insect of waxing it into the holder*

With a carefully executed preparation, no discernible adverse after-effects follow insertion of the insect into the holder. Recovery from the anaesthesia is rapid, and active locomotory movements of the limbs are seen within a minute or so. Subsequently, the insect is capable of absorbing water from subsaturated atmospheres. Electrobalance recordings indicate that this ability is fully restored within a few hours; after this initial recovery period, no impairment of this ability is seen. In this respect, the present technique does not differ from that of the experiments reported earlier (Noble-Nesbitt, 1970*a*, *b*), when the control application of wax stopped short of blocking the anus.

Two further observations also parallel the situation found in those earlier experiments. Survival of the treated insects in some cases is long term. In the present investigation, survival of unfed insects held in 85 % R.H. at 21 °C for up to 15 weeks was recorded. In other cases, completely successful moulting occurred.

These instances indicate that the technique employed does not damage the fragile insect in any major long-lasting or irreparable way.

(*b*) *Control checks with the holder arrays*

Weighings of the basic holder of capsule plus wax were carried out before and after exposure to the desiccating and hydrating conditions employed in the main experiments. No measurable weight changes occurred. Changes in weight of the capsule and wax during the experimental runs were therefore ignored. All of the weight changes were attributed to changes in weight of the insect, and were taken to represent changes in the water content of the insect.

Previously desiccated, intact insects were loosely confined in the closed chambers of the complete arrays. No weight gains occurred during exposure of the complete arrays to the hydrating conditions of 83 % R.H. at 37 °C as used in the main experiments, indicating the satisfactory nature of the sealing of the chambers. The same insects when released showed normal weight gains on exposure to these hydrating conditions.

When hydrated insects were confined in the closed chambers of the complete arrays, which were then exposed to desiccating conditions over dry calcium chloride at 37 °C, losses in weight of the insects were very low, very much below that shown when the same insects were exposed directly to the desiccating conditions, and at a level which could be accounted for by normal metabolism (Noble-Nesbitt, 1969). This again indicated the satisfactory nature of the sealing of the chambers under these conditions.

The effectiveness of the sealing of the chambers was further checked by monitoring the R.H. within the chambers using suitable salt crystals. For this purpose, clear perspex tubing was used to form the chambers, and a salt crystal was enclosed within each chamber on a polythene plate. Both dry and moist crystals were used in separate arrays, which were exposed to a variety of ambient humidities, from dry air to 85 % R.H., at 21 and 25 °C. The crystals were observed microscopically for evidence of gain

or loss of water, in high and low ambient humidities respectively. Since R.H.s between 50 and 65 % could be expected to be produced by the insect, most tests were conducted with sodium dichromate (52–55 % R.H.) and sodium nitrite (64–65 % R.H.), and since the experiments involved exposure to 83 % R.H. this was the preferred ambient humidity for the tests. No leakage was apparent over a 24 h period, confirming the results obtained when living insects were used as an assay.

The more flexible nature of the polythene tubing allowed the formation and sealing of the posterior chamber to be accomplished more easily than was possible with the perspex tubing. Since both materials performed satisfactorily in these preliminary tests, polythene tubing was used in preference, and perspex tubing was phased out in the main experiments.

(2) *Effects of shielding and exposing the anal region*

Previously desiccated, weighed individual insects were waxed into polythene capsules, as outlined above in the section on materials and methods. In each case, the basic component (capsule plus wax plus insect) was weighed. The whole array was then assembled by inserting the tip of the basic component into the polythene or perspex tubing, and was then transferred to a container at 37 °C in which the relative humidity was maintained either at 83 % by means of a saturated solution of KCl, or at a desiccating level with dry granular $CaCl_2$. Further weighings of the basic component were made at suitable intervals, providing a time course of weight change of the insect.

Groups of 3–12 insects were subjected to different sequences of experimental regime. Insects which during the course of the experiment exhibited signs of damage, to the wax seal or to the insect itself, were eliminated from consideration, along with insects which moulted during the course of the experiment, since moulting is known to affect the ability of the insect to absorb water from the atmosphere. The results are summarized in Figs. 2 and 3.

If the posterior chamber of the holder was left open but the anterior chamber was closed, the insects showed uptake when the array was put in a relative humidity of 83 % at 37 °C (Figs. 2A, C, 3A). When only the anterior end of the holder was left open, weight losses in 83 % R.H. were recorded. When the posterior end was then opened and the anterior end closed, uptake was recorded in 83 % R.H. (Figs. 2B, 3B).

Some preparations, in which both the insect and the array remained intact at the end of the first period of rehydration, were then exposed to desiccating conditions for two or three days, reweighed and again exposed to hydrating conditions, but this time in the reverse sequence, i.e. the posterior end was left open first when previously the anterior end had been left open first, and vice-versa. During this second cycle, some preparations were again exposed to a temperature of 37 °C; others were desiccated at 25 °C and rehydration was carried out at 21 °C. The results were substantially the same as before: uptake took place only when the posterior end of the insect was exposed directly to the atmosphere of high humidity, irrespective of the temperature regime in this second cycle (Figs. 2C, 3A, B).

These results clearly show that uptake is possible only when the anal region of the insect is exposed to an atmosphere of high humidity. Other constraints on the insect are not altered in any way.

In further experiments, similar results were obtained. In a few cases, however

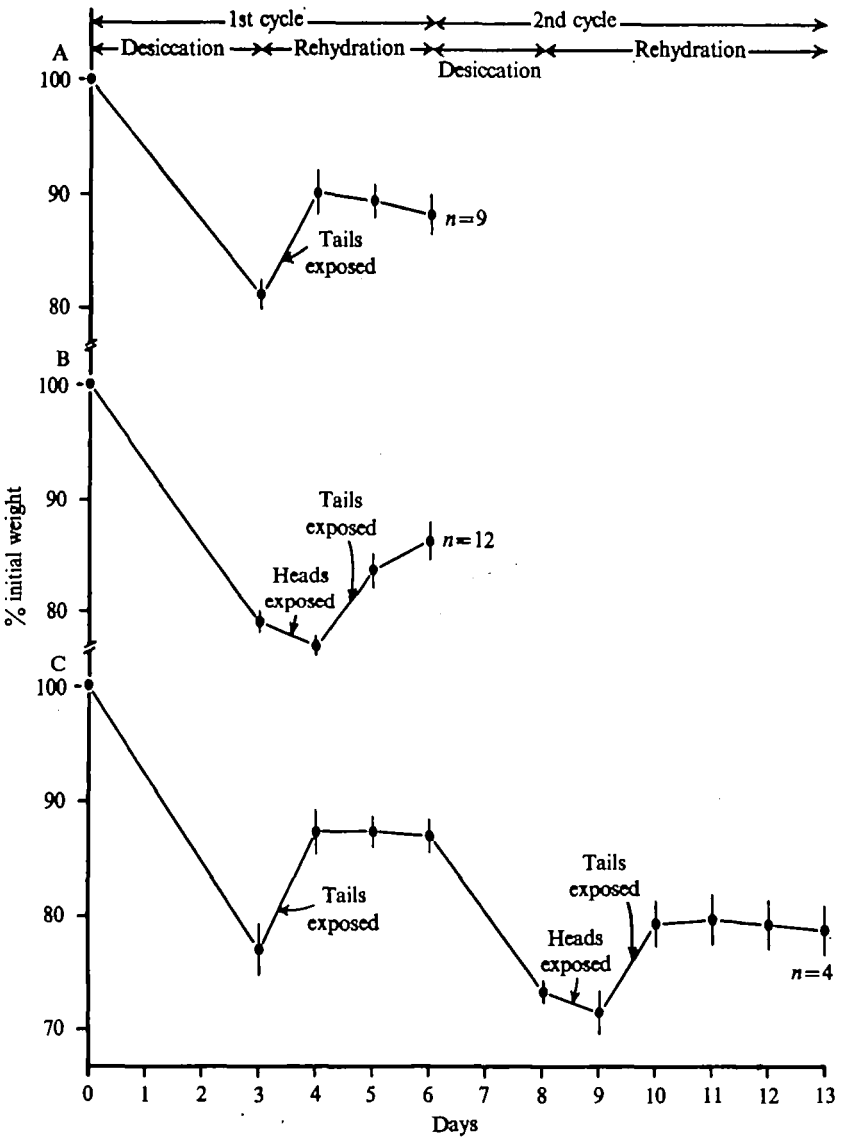

Fig. 2. The effect of exposing the tail-end and head-end of desiccated insects mounted in double-chambered arrays to 83 % R.H. at 37 °C. The insects were mounted in the double-chambered arrays at the end of the first period of desiccation. (A) A single cycle of desiccation for 3 days, followed by exposure of the tail-end only to 83 % R.H. (note the rapid uptake). (B) A single cycle of desiccation for 3 days, followed by exposure of the head-end only to 83 % R.H. for 1 day (note the small loss), and then of the tail-end only (note the rapid uptake). (C) A double cycle, with the first cycle of desiccation for 3 days, followed by exposure of the tail-end only to 83 % R.H. (note the rapid uptake); and the second cycle of desiccation for 2 days, followed by exposure of the head-end only to 83 % R.H. for 1 day (note the small loss), and then of the tail-end only (note the rapid uptake). Mean values are plotted, and the vertical lines show the standard error of the mean. The number of insects subjected to each sequence is given at the end of each trace. The lines joining the means indicate the time course of weight change.

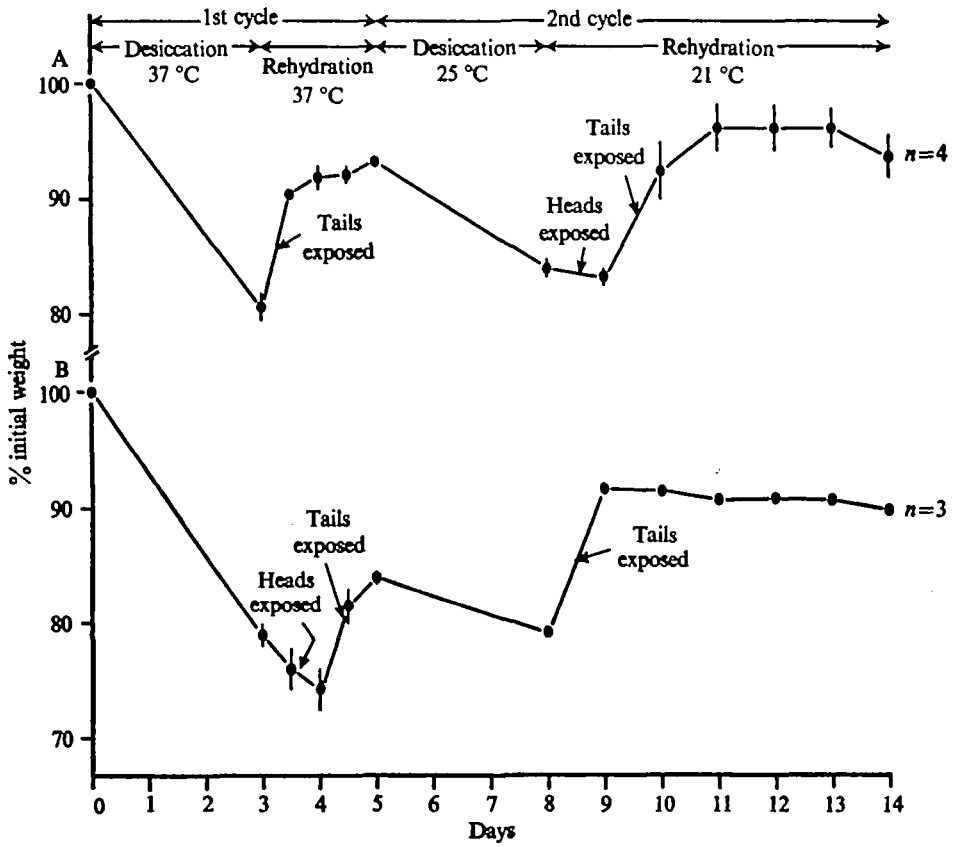

Fig. 3. Double cycles of exposure of the tail-end and head-end of desiccated insects mounted in double chambered arrays to atmospheres of high humidity, at different temperatures. The insects were mounted in the double-chambered arrays at the end of the first period of desiccation. (A) First cycle of desiccation at 37 °C for 3 days, followed by exposure of the tail-end only to 83 %R.H. at 37 °C (note rapid uptake); second cycle of desiccation at 25 °C for 3 days, followed by exposure of the head-end only to 85 % R.H. at 21 °C (note small loss), and then of the tail-end only (note rapid uptake). (B) First cycle of desiccation at 37 °C for 3 days, followed by exposure of the head-end only to 83 % R.H. at 37 °C (note small loss), and then of the tail-end only (note rapid uptake); second cycle of desiccation at 25 °C for 3 days, followed by exposure of the tail-end only to 85 % R.H. at 21 °C (note rapid uptake).

small weight gains of less than 1 mg per day occurred when only the anterior end was open. This compares with the high rate of 7·5 mg per day obtained with some insects with the posterior end open, and it probably resulted from slow leakage past the seal in these cases (see Discussion).

(3) *Continuous weight recordings*

The effect of anal shielding described in the previous section is completely and immediately reversible, as can be seen from continuous weight recordings made with individual insects, using the electrobalance technique. This technique provides accurate monitoring of the rate of gain or loss over very short periods, thus allowing repeated reversals to be carried out sequentially with a single insect.

A specimen recording made during an experimental run on an insect is shown in

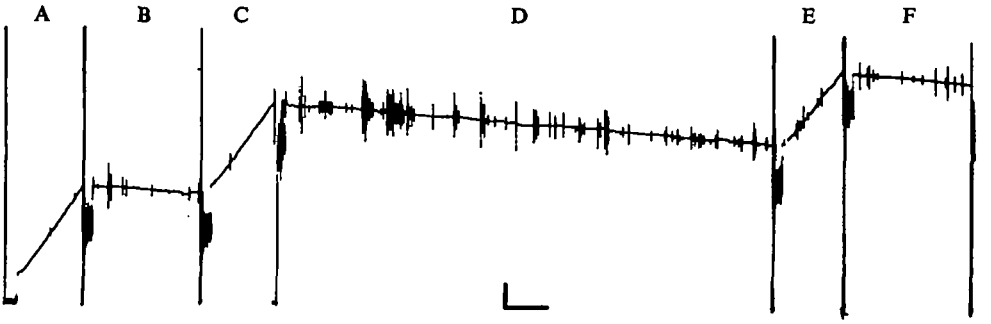

Fig. 4. Electrobalance recording of three cycles of reversal of tail-end and head-end exposure of a previously desiccated insect mounted in a double-chambered array to 85 % R.H. at 26 °C. (A) First cycle, tail-end exposed. Note immediate, rapid, and continuous uptake, with little evidence of locomotor activity. (B) First cycle, head-end exposed. Note continuous slow loss, with much greater locomotor activity. (C) Second cycle, tail-end exposed. Immediate, rapid and continuous uptake with little locomotor activity. (D) Second cycle, head-end exposed. Continuous slow loss, with much locomotor activity, over an extended period. (E) Third cycle, tail-end exposed. Immediate, rapid and continuous uptake, with little locomotor activity. (F) Third cycle, head-end exposed. Continuous slow loss, with some locomotor activity. The small-scale vertical excursions of the trace indicate locomotor activity. The large vertical excursions mark the beginning and end of each phase of the recording, when the insect was removed from the pan of the balance to allow reversal of the capping, and the trace stopped. At the beginning of each phase, the 10 min period of air stirring is shown by the gross depression and increased 'noise' level of the trace. Scales: *ordinate*, 0·1 mg; *abscissa*, 1 h.

Fig. 4, which shows the first three cycles of exposure of this insect. The insect had previously been desiccated for 3 days, sealed into the double-chambered array, allowed to rehydrate fully to check its functioning, and desiccated again for 3 days. It was then introduced into the temperature–humidity chamber below the electrobalance and placed on the pan of the balance. The magnetic fan was activated for approximately 10 min. after closing the temperature–humidity chamber, to bring about rapid equilibration. Saturated KCl solution was used to produce a high humidity, of 85 % R.H. at the temperature of 26·5 ± 1·0 °C at which the experiment was conducted. During the first cycle, the first part (A) of the recording was obtained whilst only the posterior chamber of the array was uncapped, allowing direct access of the humid atmosphere to the anal region of the insect. Uptake of water from this sub-saturated, but humid, atmosphere, as shown by increase in weight, commenced immediately, and was rapid (at a rate equivalent to over 5 mg/day) and continuous. Active movements of body and limbs were rare during this phase.

This phase of uptake was ended by removing the insect array from the balance chamber. The posterior chamber of the insect array was capped, and the anterior chamber uncapped. The wax seals were checked, and the insect was returned to the balance. A 10 min period of air-stirring followed the closing of the temperature-humidity chamber and any necessary resetting of the balance was carried out. Part B of the recording was thus obtained with only the anterior part of the body directly exposed to the humid atmosphere. A slight overall loss resulted during this phase, at a rate of less than 0·017 mg/h, which is equivalent to less than 0·4 mg/day. Body and limb movements were more noticeable.

Similar results were obtained during the second and third cycles (Fig. 4C-F).

The pattern was repeated during subsequent cycles with this insect, though in the later cycles uptake did not always commence immediately when the tail-end was exposed. During the final phase of the experiment, with the tail-end exposed, uptake proceeded until an equilibrium level was reached, when slight and gradual fluctuations about this level began; latterly the overall trend was a slow loss, probably reflecting dry weight losses from continuing metabolic activity (cf. Noble-Nesbitt, 1969).

Other experiments confirmed this pattern over a range of ambient temperatures (21–27 °C). In a few experiments, slow uptake occurred when the posterior chamber was capped. In most of these cases, leaks were readily traced to cracks which had formed in the wax seals; when the seals were made good, the effect was eliminated, indicating that leakage past the seal was the probable cause of this effect.

Lack of gain in weight when both chambers were capped confirmed the satisfactory nature of this method of closing the chambers.

With both chambers open, the rate of uptake was similar to that of the same preparation with only the tail-end open. Comparison of the rates of gain with the tail-end open (approximately 250 μg/h or 6·0 mg/day) and of loss with the head-end open (approximately 12·5 μg/h or 0·3 mg/day) shows that losses from the head-end would only marginally affect net uptake, within the limits of variation of rate of uptake in an individual insect.

(4) *Relative humidity within small closed chambers*

Intact insects taken direct from culture conditions and enclosed individually within small tubes, approximately 1 ml in capacity, held at 21 °C, usually produced and maintained 60–65 % R.H. (as determined by observation of salt crystals placed in the tubes) so long as they remained alive (often for periods of many days). Usually, sodium nitrite (65 % R.H.) was completely precipitated whilst sodium dichromate (52–55 % R.H.) went into solution. Some fluctuation in the R.H. with time was observed with individual insects, indicating the operation of an overall mechanism controlling net gain or loss of water, for example possibly by means of the turning on or off of rectal uptake. Similar results were obtained with other fully hydrated intact insects previously subjected to a period of starvation whilst being held in high humidity (83 % R.H.), or whilst being subjected to a cycle of desiccation and rehydration. Intact desiccated insects were able to produce even lower R.H.s (down to about 50 %), removing water from sodium dichromate (52–55 % R.H.) but losing it to potassium nitrate (45–48 % R.H.) in some cases. This agrees closely with the results obtained by Beament *et al.* (1964), who showed that uptake occurs in this insect down to 45 % R.H.

Desiccated insects inserted in the double-chambered array with a fully effective seal between the chambers were also able to lower the R.H. in the tail-end chamber to about 50 %, and whilst doing this the anal valves could be seen to move, indicating the operation of the rectal uptake mechanism (Noble-Nesbitt, 1973). Such low R.H.s were not produced in the head-end chamber, but humidity rose only slowly, a reflexion of very low rates of evapotranspiration from the insect. In some cases, no difference was found between the two chambers, and in these cases small-scale uptake occurred on exposure of the head-end alone to high humidity, indicating that the seal between the chambers was not fully effective. In these cases, rectal uptake evidently kept the humidity low in both chambers.

These results show that firebrats confined in small sealed chambers are able to take up water from the atmosphere over prolonged periods, and that the anal region of the insect is responsible for this function. It is quite evident that when the tail-end chamber is sealed, the uptake capability of the anal region is retained, but without access to a larger volume of humid air, uptake of water ceases when the R.H. of the sealed tail-end chamber falls to the critical equilibrium level of approximately 50%. The small amount of water gained by the insect by this lowering of the R.H. in the tail-end chamber is insufficient to register on the balance, amounting to less than 0·02 mg.

DISCUSSION

The anal region of *Thermodia domestica* is clearly implicated in the absorption of water from subsaturated atmospheres. This interpretation, first advanced on the basis of clear-cut results from blocking experiments (Noble-Nesbitt, 1970*a*, *b*), is further confirmed by the results from the investigations reported here.

The isolation of the anterior parts of the insect's body from the anal region, allowing these two regions to be exposed independently to the surrounding atmosphere, has been effected by sealing the insect into an aperture separating two small chambers capable of being opened or closed independently of one another. A completely effective seal is difficult to achieve in all cases; because of the longitudinal and lateral overlapping of parts of the insect's body it is virtually impossible to eliminate completely channels linking the two chambers. In a tightly-fitting seal, however, the effect of these channels is reduced to insignificant proportions and any residual 'leakiness' does not give rise to any measurable effect. As we have seen, in a few preparations a small residual leak was evidently present, giving rise to a measurable but small effect. The amount of water apparently transferred in this way added up to about 1 mg/day. Slow diffusion along the restricted channels formed by the body folds could perhaps account for this effect.

Diffusion of water in the vapour phase depends partly upon the vapour pressure difference, the length of the diffusion pathway and the cross-sectional area of this diffusion pathway. With a relative humidity of 83% in the anterior chamber, a vapour pressure difference equal to 33% of the saturated water vapour pressure can be assumed between the anterior and posterior chambers, since *Thermobia* is able to take up water from atmospheres with humidities just below 50% R.H. (Beament *et al.*, 1964; present results). The length of the diffusion pathway along the body from one side to the other of the wax seal is approximately 1 mm. For a diffusion rate of 1 mg/day, these values suggest a cross-sectional area of about 0·05 mm^2 for the diffusion pathway, a small value commensurate with the pathway which could be formed by the body folds.

As a further possibility, unidirectional ventilatory air-flow through the longitudinal trunks of the tracheal system in an antero-posterior direction could perhaps account for the transfer of water vapour from the anterior chamber to the posterior chamber, where it would be extracted from the atmosphere by the rectum. Air entering the tracheal system anteriorly at 83% R.H. would most likely become saturated with water vapour (Weis-Fogh, 1967), so that it would emerge in the posterior chamber fully saturated. This would represent a loss of water from the insect. However, if the air were then dried down to 50% R.H., this loss would be more than recovered. The net

gain of water from the air would be equal to the amount extracted in lowering the humidity from 83% R.H. (as in the anterior chamber) to 50% R.H. This represents 33% of the water which air can hold at that temperature when fully saturated. Since 1 ml of fully saturated air at 37 °C contains 0·045 mg water vapour, 1 ml of air will supply 0·015 mg water vapour to the insect. A ventilatory flow of less than 100 ml air/day would supply 1 mg of water to the insect. This approximates to a ventilatory flow of 100 l of air/kg body wt/h, a value between the resting (40 l/kg/h) and active (450 l/kg/h) ventilation rates of locusts (Weis-Fogh, 1967). Since the rectal tissue is presumably metabolically very active (Maddrell, 1971), and can therefore be expected to augment the general respiratory requirements of the insect (as does the flight musculature of flying locusts), this value would seem a reasonable one. It is not known whether unidirectional ventilation occurs in *Thermobia* or not, but even if it does, it would have to be assumed that such unidirectional ventilation occurs only in some insects, since otherwise small-scale leakage would be found in all preparations, instead of only in a few. Further, the volume of the tail-end chamber is less than 1 ml and it is therefore incapable of accepting a net gaseous inflow of 100 ml during the course of a day, as unidirectional ventilation would require. It seems likely, therefore, that the more probable explanation of this small-scale uptake is that of the body-fold diffusion pathway.

What is clear from the results reported here, however, is that large-scale uptake occurs only when the tail-end of the insect is exposed directly to the atmosphere of high humidity. When the anal region of the insect is not exposed to high humidity, and the seal separating the two ends of the insect's body is fully effective, then no matter how much of the remainder of the body is exposed to high humidity, only losses occur. When the seal is apparently fully effective, the change from a water-loss situation when only the head-end is exposed to high humidity, to a water-gain situation when only the tail-end is so exposed, is immediate and reversible. The electrobalance recordings show this especially well. This reversible arrest of uptake is achieved without altering the constraints on the insect in any way other than shielding the anal region from, or exposing it to, high R.H., a procedure which does not affect the functioning of the rectal region, as shown by the lowering of the humidity within the sealed tail-end chamber to about 50% R.H. and by the continued movements of the anal valves. The suggestion by Okasha (1971) that the arrest of uptake following anal blockage may be mediated by interference with the volume regulatory system of the insect is not substantiated by these results.

With the tail exposed, uptake continues until the water lost during desiccation is replenished, making due allowance for the continuing effects of starvation, which is known to result in an overall weight loss associated with a higher degree of hydration, an effect which has been attributed to the regulation of volume (Noble-Nesbitt, 1969; Okasha, 1971, 1972). This effect is clearly seen in Figs. 2 and 3, and was evident in the longer term electrobalance runs, as described above. As we have seen, when the insect is already near its equilibrium level, uptake does not always begin immediately on exposing the tail-end to a high humidity, indicating that the situation is less stressful for the insect (as reflected in its delayed response). In turn, this perhaps gives some indication of the operation of its physiological control system, which apparently is tighter and responds more rapidly in the more extreme situations of water stress.

These experiments demonstrate conclusively that for water uptake to occur in *Thermobia*, an atmosphere with a sufficiently high R.H. must be presented directly to the anal region of the insect. Other lines of the investigation, dealing with the behaviour of this region of the insect and with the structure and functioning of the posterior sacculate region of the rectum in relation to atmospheric water absorption, also indicate that in *Thermobia* the posterior sacculate region of the rectum must be freely in contact with an atmosphere of high humidity for uptake of water vapour to occur.

What remains to be shown is the mechanism by which water vapour entering along this avenue is taken up by the insect's tissues. In ticks, production of a solution rich in potassium and sodium seems to be involved (Rudolph & Knülle, 1974), though the precise mechanism by which solutions of sufficiently high concentration are produced and the water taken up by them from the air made available to the tissues remains unclear. In *Thermobia* which has an even lower critical equilibrium humidity of below 50% R.H., the gradient and therefore the problem is even greater (Noble-Nesbitt, 1973). Here, too, the elucidation of the precise mechanism of uptake must await further investigation.

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